



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

I-010697-P-0069

APR 19 2007

• U.S. Department of Interior
Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Attention: David Erdahl, Ph.D.
Branch Chief
4050 Bridger Canyon Road
Bozeman, MT 59715

Re: Request to review Effectiveness final study report (FLOR-01-EFF.3-27) and the Effectiveness technical section for AQUAFLO

Dear Dr. Erdahl:

Based on the information in your submission dated November 29, 2006, and amended on January 11, 2007, and the information contained in the Investigational New Animal Drug (INAD) file 010697, the Division of Therapeutic Drugs for Food Animals considers the Effectiveness technical section for AQUAFLO (florfenicol) Type A Medicated Article for the control of mortality in freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida*, when administered at a dose of 10 mg/kg of fish/day for 10 consecutive days to be complete.

We will make a final decision on whether we can approve your application after we have reviewed all of the data for all applicable technical sections submitted in support of an Administrative New Animal Drug Application (NADA), NADA, or supplemental NADA, and any other information available to us, as a whole, and determined whether the requirements for approval set forth in the Federal Food, Drug, and Cosmetic Act have been met.

If you submit correspondence relating to your submission to the investigational file, you should reference this letter by date and the principal submission(s) identifier found at the top of this letter. If you have any questions, please contact me at (301) 827-7571 or Dr. Donald Prater, Leader, Aquaculture Drugs Team, at (301) 827-7567.

Sincerely,

for
Joan C. Gotthardt, D.V.M.
Director, Division of Therapeutic
Drugs for Food Animals
Office of New Animal Drug Evaluation
Center for Veterinary Medicine

Enclosure: Freedom of Information Summary (Effectiveness section)

II. EFFECTIVENESS

A. Dose Characterization

Aeromonas salmonicida is the bacterial pathogen associated with furunculosis in freshwater-reared salmonids. This disease is frequently present as a systemic disease that may result in high levels of mortality in affected fish.

The florfenicol dose selected for the effectiveness trials was based on the *A. salmonicida* minimum inhibitory concentration of florfenicol, the pharmacokinetic profile of florfenicol in salmonids, and the results of field studies for saltwater-reared salmonids available in the published literature.

Based on that information, florfenicol administered at a dose of 10 mg/kg of fish daily had the potential to control furunculosis outbreaks associated with *A. salmonicida* in freshwater-reared salmonids.

B. Substantial Evidence

The results of the three following studies, when considered together, demonstrate that florfenicol is effective when administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days for the control of mortality in freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida*.

1. Field Study

- a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fingerling Coho Salmon, *Oncorhynchus kisutch*, Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*"
(Study Number FLOR-01-EFF-01)
- b. Investigator: Al Jensen
Makah National Fish Hatchery
U.S. Fish and Wildlife Service
Neah Bay, WA
- c. Study Design:
 - 1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality in fingerling coho salmon due to furunculosis associated with *A. salmonicida*.
 - 2) Study Animals: Approximately 2,400 fingerling coho salmon
 - 3) Treatment Groups: The study included two treatment groups with six replicates of each treatment. Each replicate was a tank of fish. Treatments were assigned to tanks using a completely randomized study

design. One florfenicol-treated tank was removed from the study because of an interruption in water flow.

- 4) **Drug Administration:** Florfenicol was administered in a commercial salmonid feed at a dose of either 0 or 10 mg florfenicol/kg of fish daily. Study feeds were fed for 10 consecutive days.
- 5) **Measurement and Observations:** *A. salmonicida* was identified on cultures of spleen and kidney tissue collected 5 days before the start of the treatment period (60 fish), and on the first day of the treatment period (54 fish). The clinical signs observed were consistent with a furunculosis infection. Approximately 220 fish were randomly transferred to the study tanks. The study included a one-day acclimation period, a 10-day treatment period, and a 7-day post-treatment period. Dead fish (mortalities) were counted and recorded twice daily. On Day 18, six fish were collected from each tank for examination and collection of samples for culture. Fish examinations on Day 18 revealed an infection with the external parasite, *Trichodina* sp. The study was ended and treatment for the parasite infestation was initiated.
- 6) **Statistical Analysis** The design of this study precluded statistical analysis.

- d. **Results:** Mortality results are included in the following table.

Table 1. Mortality results for a field effectiveness study in coho salmon with a 10-day treatment period and 7-day post-treatment period

Florfenicol Dose (mg/kg of fish)	Percent Cumulative Mortality
0	30.3 (331/1,092)
10	11.1 (145/1,306)

- e. **Adverse Reaction:** No adverse reactions were reported in this study.
- f. **Conclusion:** Results of this study support the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in coho salmon, *Oncorhynchus kisutch*, due to furunculosis associated with *Aeromonas salmonicida*.

2. Field Study

- a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fall Chinook Salmon, *Oncorhynchus tshawytscha*, Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*"
(Study No. FLOR-01-EFF.3-22)
- b. **Investigator:** Randy Rickert
Makah National Fish Hatchery
U.S. Fish and Wildlife Service
Neah Bay, WA

c. Study Design:

- 1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality in fall chinook salmon fingerlings due to furunculosis associated with *A. salmonicida*.
- 2) Study Animals: Approximately 1812 fall chinook salmon fingerlings
- 3) Treatment Groups: The study included two treatment groups with six replicates of each treatment. Each replicate was a tank of fish. Treatments were assigned to tanks using a randomized block study design.
- 4) Drug Administration: Florfenicol was administered in a commercial salmonid feed at a dose of either 0 or 10 mg florfenicol/kg of fish daily. Study feeds were fed for 10 consecutive days. The trial feed was assayed to confirm the florfenicol concentration.
- 5) Measurement and Observations: Fish were held in one raceway prior to allocation to study tanks. Dead, moribund, and healthy fish were collected 5 days prior to the start of the acclimation period for examination. *A. salmonicida* was identified on cultures and imprints of kidney tissue. Equivalent numbers of fish were transferred to each study tank in five rounds, for a total of approximately 151 fish per tank. The study included a one-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. Dead fish (mortalities) were counted once or twice daily. Dead fish were collected during the treatment period (31 fish) and the post-treatment period (10 fish). Kidney tissue was collected from each fish for culture. *A. salmonicida* was identified via cultures of kidney tissue collected from the fish. Fish behavior and appetite were observed daily.
- 6) Statistical Analysis: Arcsine-transformed Kaplan Meier estimates of survival were calculated for each tank. Means of treated and control tanks were then compared using a t-test. Significant differences were seen between treated and control groups immediately after treatment, after treatment plus a 10-day holding period, and after treatment plus a 14-day holding period. Mixed model repeated measures analyses using a binomial distribution with canonical logistic link showed that most of the differences in cumulative survival were due to reduced daily mortality rates among treated individuals from 4 to 18 days after the start of florfenicol administration.

- d. Results: Mortality results are included in the following table.

Table 2. Mortality results for a field effectiveness study in chinook salmon with a 10-day treatment period and 14-day post-treatment period

Florfenicol Dose (mg/kg of fish)	Percent Cumulative Mortality (Cumulative Mortality)
0	94.4 (808)
10	16.2 (141)

The treated and untreated control groups differ significantly in the cumulative percent mortality ($p < 0.001$).

- e. Adverse Reaction: No adverse reactions were reported in this study.
- f. Conclusion: Results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in fingerling fall chinook salmon, *Oncorhynchus tshawytscha*, due to furunculosis associated with *A. salmonicida*.

3. Field Study

- a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality Coho Salmon, *Oncorhynchus kisutch*, Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*" (Study No. FLOR-01-EFF.3-27)
- b. Investigator: Randy Rickert
Makah National Fish Hatchery
U.S. Fish and Wildlife Service
Neah Bay, WA
- c. Study Design:
- 1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality in coho salmon fingerlings due to furunculosis associated with *A. salmonicida*.
 - 2) Study Animals: Approximately 1920 coho salmon fingerlings
 - 3) Treatment Groups: The study included two treatment groups with six replicates of each treatment. Each replicate was a tank of fish. Treatments were assigned to tanks using a randomized block study design.
 - 4) Drug Administration: Florfenicol was administered in a commercial salmonid feed at a dose of either 0 or 10 mg florfenicol/kg of fish daily. Study feeds were fed for 10 consecutive days. The trial feed was assayed to confirm the florfenicol concentration.

- 5) **Measurement and Observation:** Fish were held in one raceway prior to allocation to experimental tanks. Dead and moribund fish were collected 2 days prior to the start of the acclimation period for examination and tissue collection for culture. *A. salmonicida* was identified on cultures of kidney tissue. Equivalent numbers of fish were randomly transferred to each study tank in four rounds, for a total of approximately 160 fish per tank. The study included a 2-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. Dead fish (mortalities) were counted once or twice daily. Moribund fish were collected during the treatment period (3 fish) and the post-treatment period (2 fish). Kidney tissue was collected from each fish for culture. *A. salmonicida* was identified via cultures of kidney tissue collected from control group fish. Fish behavior and appetite were observed daily.
- 6) **Statistical Analysis:** Arcsine-transformed proportion survival were calculated for each tank. Means of treated and control tanks were then compared using a t-test. Significant differences were seen between treated and control groups immediately after treatment, after treatment plus a 10-day holding period, and after treatment plus a 14-day holding period. Mixed model repeated measures analyses on arcsine transformed daily mortality rates showed that most of the differences in cumulative survival were due to reduced daily mortality rates among treated individuals from 8 to 19 days after the start of florfenicol administration.
- d. **Results:** Mortality results are included in the following table.
- Table 3.** Mortality results for a field effectiveness study in coho salmon with a 10-day treatment period and 7-day post-treatment period.
- | Florfenicol Dose
(mg/kg of fish) | Percent Cumulative Mortality
(Cumulative Mortality) |
|---|--|
| 0 | 29.5 (282/956) |
| 10 | 16.0 (151/946) |
- The treated and untreated control groups differ significantly in the cumulative percent mortality ($p < 0.005$).
- e. **Adverse Reactions:** No adverse reactions were reported in this study.
- f. **Conclusion:** Results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in fingerling coho salmon, *Oncorhynchus kisutch*, due to furunculosis associated with *A. salmonicida*.